

Fishery Data Series No. 91-50

Precision of Ages Determined from Six Bony Structures of Two Coregonids

by

L. Saree Timmons

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Alaska Department of Fish and Game

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ABSTRACT

Ten techniques for ageing of humpback whitefish *Coregonus pidschian* and least cisco *Coregonus sardinella* were evaluated. Four readers independently estimated the age of each fish using five whole bone structures (scales, opercles, subopercles, vertebrae, and otoliths) and five sectioned structures (fin rays, opercles, subopercles, vertebrae, and otoliths); readings were replicated three times. Based on analyses of variance and sampling standard errors scales, sectioned fin rays, and whole opercles were best for age validation studies of humpback whitefish; scales, sectioned fin rays, and whole subopercles were best for least cisco. Ages estimated from scales were not significantly lower than ages estimated from most other methods. Sectioning of most structures did not result in significantly greater ages, and offered little or no improvement in precision over whole structures. Using our data set as an example, it is shown how imprecise age estimates, regardless of accuracy, can lead to erroneous conclusions about fish stocks.

KEY WORDS: humpback whitefish, least cisco, ageing, scales, opercles, subopercles, vertebrae, otoliths, fin rays, precision, *Coregonus pidschian*, *Coregonus sardinella*.

INTRODUCTION

Ages determined from the bony parts of fish are often used to estimate age composition, growth, mortality, and recruitment. Because fishery management decisions are often based on such estimates, choosing the best method for age determination is essential. Accuracy and precision are important considerations when choosing methods of age determination. Accurate ages are true ages. Precise ages can be repeated by several readers several times. Beamish and McFarlane (1983, 1987) stressed the importance of validating the accuracy of age determination techniques by species and region, because inaccurate estimates of age can ultimately lead to serious errors in fishery management. Imprecise age estimates can also lead to serious errors. If bony structures with ambiguous markings are used for determining ages, it may be impossible to compare estimates by different readers or even by the same reader.

Bony structures such as scales, fin rays, and otoliths, have been used to determine the ages of coregonids since the early 1900's, but the precision of these techniques has not been critically examined. Van Oosten's (1923) work on the scales of lake whitefish *Coregonus clupeaformis* of known age was one of the first attempts to validate ages estimated from a bony structure. Although he concluded that ages determined from scales were accurate, precision was not addressed. In a study of annulus formation on the scales of whitefish, Hogman (1968) concluded that ages determined from scales were accurate, but found "accessory checks" that he believed could be misinterpreted as annuli. Recent investigations (Mills and Beamish 1980) concluded that fin rays were more reliable than scales for ageing lake whitefish in some situations. Otoliths were more accurate than scales for estimating the ages of lake whitefish and the cisco *Coregonus albula* (Aass 1972; Power 1978).

The need for an evaluation of precision in the ageing of humpback whitefish *Coregonus pidschian* and least cisco *Coregonus sardinella* in Alaska prompted this study. Precision and estimates of mean age were compared among ten different techniques for determining age of humpback whitefish and least cisco. The bony structures evaluated in this study included six structures, of which five were examined as whole structures (scales, opercles, subopercles, vertebrae, and otoliths) and five were examined as sectioned structures (fin rays, opercles, subopercles, vertebrae, and otoliths).

METHODS

Field and Laboratory Procedures

Humpback whitefish and least cisco were collected from the Chatanika River, Alaska (65°06'45"N, 145°28'20"W) during August and September, 1988 with an electrofishing boat and seines. After the fish were measured (fork length) and weighed, six structures were removed from each fish: scales, left opercle, left subopercle, thoracic vertebrae, sagittal otoliths, and left pectoral fin. Scales were removed from the left side of the fish from an area above the lateral line and below the dorsal fin.

Five humpback whitefish were randomly chosen from each of six length groups (340-359, 360-379, 380-399, 400-419, 420-439, 440-459 mm), except the 440-459 mm group, from which only four fish were available, for a total of 29 fish. Five least cisco were randomly selected from each of seven length groups (< 300, 300-309, 310-319, 320-329, 330-339, 340-349, > 349 mm) for a total of 35 fish.

A variety of preparation methods were used for the different bony structures. Scales were scrubbed clean with a toothbrush, hot water, and detergent. Two scales from each fish were impressed on acetate cards with a Carver hydraulic press at a temperature of 93°C and pressure of 137,895 kPa for 30 seconds. Opercles, subopercles, vertebrae, and fin rays were simmered in hot water (just under 100°C) with detergent and then cleaned of all remaining tissue. Three to four center fin rays were removed from each pectoral fin. The surface of one otolith from each pair was ground on a water-wetted Carborundum stone to enhance readability (after Nordeng 1961). All structures were stored dry in coin envelopes until examination. Impressions of scales were viewed with a microfiche reader at about 32×. Whole opercles, subopercles, vertebrae, and otoliths were examined against a dark background with the aid of a dissecting microscope at 10× and 40×. Opercles, subopercles, and vertebrae were viewed dry; otoliths were viewed in glycerin. Fin rays were not examined whole.

After examination of whole structures was completed, opercles, subopercles, vertebrae, otoliths, and fin rays were sectioned. These structures were embedded in clear epoxy and then sectioned with the emery cut-off wheel of a Dremel Moto-Tool® (Temple et al. 1985). Opercles were sectioned through the thickened basal center, perpendicular to the surface ridges, and the thickened basal tips of the subopercles were cross-sectioned. Procedures for sectioning opercles and subopercles were not available, so several trial specimens were sectioned to determine the angle at which markings, similar to those on sectioned fin rays (Mills and Beamish 1980), were visible. Vertebrae were cross-sectioned through the center of the centrum, from rim to rim (after Prince et al. 1985), while otoliths were halved laterally through the nucleus (Beamish 1979). Fin rays were cross-sectioned close to the base (after Mills and Beamish 1980). From each opercle, subopercle, and fin ray, three or four sections, approximately 1 mm thick, were cut. The best section from each structure and fish, as determined by visibility of markings, was chosen for inclusion in the study, but both halves of each vertebra and otolith were used. Therefore, readers had only one opercle, subopercle, and fin ray section from each fish to examine, but two vertebra and otolith sections. All sections were mounted to glass slides with glue, and were examined through a compound microscope at about 80×.

Each reader examined each set of structures three times (humpback whitefish: 10 structures × 29 fish × 4 readers × 3 replicates = 3,480 readings; least cisco: 9 structures × 35 fish × 4 readers × 3 replicates = 3,780 readings). Order of structure and fish to be read was determined randomly, but each reader finished a replicate across all structures before beginning the next replicate, and within each replicate readings of one structure were completed before beginning the next structure. Readers recorded, by structure, the time taken to complete each set of structures within each replicate.

Criteria for counting annuli on whole structures have been well-defined. Closely spaced circuli, sometimes accompanied by crossing-over, were considered annuli on scales (Van Oosten 1923; Figure 1). Opercular and subopercular annuli were defined as concentric opaque ridges formed on the surface of the structure, and were counted outwards from the thickened basal center (McConnell 1952). Annuli on vertebrae were defined as thin, slightly translucent ridges found in the centrum (Appelget and Smith 1950); on otoliths, translucent circular bands radiating from the nucleus outward, were considered annuli (Beamish 1979).

Annuli on sectioned structures were defined according to previously published criteria when available. On vertebral sections, translucent bands, alternating with dark-appearing opaque bands, were considered annuli (Prince et al. 1985). Although narrow at the center of the section, these translucent bands became wider towards the rim, with some being the same width as the opaque bands. Annuli on sectioned otoliths and fin rays were defined as thin translucent rings radiating outward from the nucleus (Beamish 1979; Mills and Beamish 1980). Existing criteria for annuli of sectioned opercles and subopercles were not available. Therefore, annuli on opercle and subopercle sections were defined as thin, translucent bands radiating from the center of the thickened basal area outwards to the anterior edge, similar to definitions of annuli on sectioned fin rays (Mills and Beamish 1980; Figure 1). Although vertical striations seen on the surface were visible on the sections, the surface annuli were not visible. Of the 12 sectioned subopercles from least cisco examined in a pre-test, nine (75%) had no distinguishable annuli. Therefore, sectioned subopercles from least cisco were eliminated from further consideration.

Four readers with a variety of experience at age determination were chosen to participate in the study. Each reader was instructed on the different methods of age determination before commencing the readings. Reader 1 had some experience with all of the whole structures, reader 2 had no experience at determining the ages of fish, reader 3 had no experience with whitefish but had previously determined ages of other species from scales and otoliths, and reader 4 had determined whitefish ages with scales and ages of other species of fish with otoliths. None of the readers had previously used sectioned bone structures in determining the ages of fish.

Statistical Analysis

Means and precision of estimated ages were compared among structures. Means of estimated ages were calculated for combinations of structures and readers, and for individual structures. An analysis of variance (ANOVA) was used to detect significant differences between the estimates of mean ages from the ten methods and four readers (Neter et al. 1990). The ANOVA was a balanced design with structures as fixed effects, readers as random effects, and fish as an exogenous source of variation. During the study a small number of specimens was broken or lost. Because of the large sample size, performing an ANOVA with an unbalanced design was not feasible. Therefore, the balance of the design was maintained by inserting marginal means for missing data into the analysis and appropriately decreasing the degrees of freedom in hypothesis

$$SSE_{kl} = \left[\frac{\sum_j^f \sum_i^n (X_{ijkl} - \bar{X}_{jkl})^2}{f(n-1)} \right]^{1/2}; \quad (3)$$

where f is the number of fish examined. The SSE_{kl} in equation 3 is analogous to the absolute percent error of Beamish and Fournier (1981) and can be used to measure the error due to the k th reader in determining ages from the l th structure.

To compare precision of structures overall, sampling standard errors by structure (SSE_l) were calculated:

$$SSE_l = \left[\frac{\sum_k^r \sum_j^f \sum_i^n (X_{ijkl} - \bar{X}_{jkl})^2}{rf(n-1)} \right]^{1/2}. \quad (4)$$

Precision of individual readers (SSE_k) was calculated with the equation:

$$SSE_k = \left[\frac{\sum_l^s \sum_j^f \sum_i^n (X_{ijkl} - \bar{X}_{jkl})^2}{sf(n-1)} \right]^{1/2}, \quad (5)$$

where s is the number of structures.

Sampling standard errors by fish and structure (SSE_{jl}) were regressed against length of fish to determine if precision was related to length. Times required to determine ages from each structure were compared with Friedman's test (Conover 1980), using structures as treatments and readers as blocks. Because reading time decreased with practice, only the third replicate was used for the Friedman's tests.

RESULTS

Mean estimated ages of humpback whitefish were significantly different (ANOVA, $P < 0.01$; Table 1). Although interactions were significant, the MSE's for the interactions were very small compared to the MSE's for the main effects. Estimated mean ages determined from sectioned subopercles were significantly lower than mean ages determined from the other structures (Table 2). Estimated mean ages obtained by readers 1 and 3 were not significantly different, but were significantly different from ages determined by both readers 2 and 4, and ages determined by readers 2 and 4 were significantly different from each other (Least Significant Difference Test, overall $\alpha = 0.05$).

Table 1. Results of the ANOVA for humpback whitefish and least cisco.

Source	DF	ANOVA SS	F-Value	P-Value
<u>Humpback Whitefish</u>				
Intercept	1	116,944	99999.99	< 0.01
Fish	28	3,884	185.66	< 0.01
Reader	3	920	410.29	< 0.01
Structure	9	874	129.94	< 0.01
Reader*Structure	27	861	42.69	< 0.01
Reader*Fish*Structure	1,092	3,780	4.63	< 0.01
<u>Least Cisco</u>				
Intercept	1	85,217	99999.99	< 0.01
Fish	34	780	37.13	< 0.01
Reader	3	1,428	770.57	< 0.01
Structure	8	456	92.20	< 0.01
Reader*Structure	24	385	25.94	< 0.01
Reader*Fish*Structure	1,192	2,001	2.72	< 0.01

Table 2. Mean estimated ages of humpback whitefish and least cisco determined from ten structures by four readers. Underlined values are significantly different ($P < 0.05$).

Reader	Whole Structures ^a					Sectioned Structures ^a				
	SC	OP	SO	VE	OT	FR	OP	SO	VE	OT
Humpback Whitefish										
1	5.83	6.66	6.84	7.55	6.62	5.74	6.15	4.49	6.24	5.17
2	5.60	6.23	6.00	6.54	5.84	5.53	5.90	5.08	5.94	6.02
3	5.56	6.53	6.31	5.62	6.58	6.11	5.95	4.49	6.91	8.31
4	5.49	5.50	4.99	5.25	5.00	5.08	5.06	3.67	4.80	4.58
Pooled	5.62	6.23	6.04	6.24	6.01	5.61	5.76	<u>4.43</u>	5.97	6.02
Least Cisco ^b										
1	4.41	4.77	5.10	4.87	4.84	5.06	5.75	-	4.98	5.89
2	4.86	5.18	4.98	5.35	5.09	5.35	5.21	-	5.58	5.96
3	4.01	5.23	5.39	5.12	4.29	4.72	3.96	-	5.30	6.35
4	3.92	3.93	3.91	3.64	3.36	3.65	3.45	-	3.37	4.20
Pooled	4.30	4.79	4.86	4.75	4.40	4.70	4.58	-	4.81	<u>5.60</u>

^a SC = scales, OP = opercles, SO = subopercles, VE = vertebrae, OT = otoliths, FR = fin rays.

^b Sectioned subopercles of least cisco were omitted from the study.

Ages for humpback whitefish estimated from scales were the most precise, while whole vertebrae, sectioned otoliths, and whole otoliths were the least precise (Table 3). Ages determined from sectioned opercles, vertebrae, and otoliths were only slightly more precise than ages determined from the corresponding whole structures. Thirty-four (85%) of the 40 structure-reader combinations for humpback whitefish had SSE_{kl} values less than 1.00, the smallest increment of age measured in this study. Scales, whole subopercles, whole vertebrae, sectioned fin rays, sectioned opercles, and sectioned vertebrae had significant relationships between SSE_{jl} and length ($P < 0.05$; Figure 2). Reading time was significantly different between structures ($P = 0.03$), ranging from 33 minutes per replicate for fin rays to 59 minutes per replicate for whole otoliths.

Estimated mean ages for least cisco were significantly different (ANOVA, $P < 0.01$; Table 1) Although interactions were significant, the MSE's for the interactions were very small compared to the MSE's for the main effects. Mean ages determined from sectioned otoliths were significantly greater than mean ages determined from the other structures (Table 2). All mean estimated ages by reader were significantly different from each other (Least Significant Difference Test, overall $\alpha = 0.05$). The estimated mean ages determined by reader 4 were lower than those determined by the other readers for every structure (Table 2).

All structures had SSE_l values under 1.00, ranging from 0.53 for scales to 0.98 for whole vertebrae (Table 3). Ages determined from sectioned opercles and vertebrae were slightly more precise than those determined from whole opercles and vertebrae, but ages determined from whole otoliths were more precise than those determined from sectioned otoliths. Twenty-seven (75%) of the 36 structure-reader combinations had SSE_{kl} values less than 1.00. SSE_{jl} increased significantly with increasing length for whole otoliths only ($P = 0.03$; Figure 3). Reading time was significantly different between structures ($P = 0.02$), ranging from a mean of 36 minutes per replicate for sectioned opercles to 67 minutes per replicate for sectioned vertebrae.

DISCUSSION

Precision of age estimates can aid researchers in selecting a bony structure for age validation, a time consuming and costly process. In the early stages of a validation study precision data can be used to eliminate from further consideration structures that have little potential for age determination. In this study, scales and fin rays offered the most promise for age validation studies of humpback whitefish because, although inversely related to length, precision was best overall for scales and fin rays. Whole opercles should also be considered for determining ages of humpback whitefish because precision was good and mean age was not significantly different. Scales and sectioned fin rays were also best for age validation studies of least cisco. Precision was good and did not decrease with length. Scales and sectioned fin rays gave the lowest mean ages, but not significantly so. Whole subopercles, for which precision did not decrease with length, were also good for ageing least cisco.

Table 3. Sampling standard errors of ages, by structure-reader combinations, by structure, and by reader, of humpback whitefish and least cisco determined from ten structures by four readers^a.

Reader	Whole Structures					Sectioned Structures					Totals		
	SC	OP	SO	VE	OT	FR	OP	SO	VE	OT	All	Whole	Section
Humpback Whitefish													
1	0.57	0.86	0.88	0.90	1.02	0.51	0.71	0.49	0.65	0.62	0.74	0.86	0.60
2	0.70	0.64	0.66	0.98	0.90	0.92	0.65	0.77	0.77	1.14	0.83	0.79	0.87
3	0.80	0.58	0.77	1.34	1.69	0.67	0.54	0.59	0.84	1.36	0.99	1.11	0.85
4	0.48	0.86	0.49	0.59	0.81	0.65	0.96	1.00	0.87	0.81	0.77	0.67	0.87
Pooled	0.65	0.75	0.71	0.99	1.16	0.70	0.73	0.74	0.78	1.02			
Least Cisco ^b													
1	0.38	0.45	0.56	0.57	0.70	0.57	0.98	-	0.66	1.26	0.69	0.54	0.84
2	0.67	1.16	0.90	1.02	1.06	0.52	0.60	-	0.66	0.77	0.83	0.98	0.61
3	0.54	1.02	0.84	1.48	1.01	0.51	0.65	-	0.80	1.29	0.93	1.02	0.79
4	0.50	1.09	0.67	0.51	0.59	0.67	0.83	-	0.99	0.79	0.75	0.70	0.80
Pooled	0.53	0.90	0.75	0.98	0.86	0.57	0.77	-	0.79	0.93			

^a SC = scales, OP = opercles, SO = subopercles, VE = vertebrae, OT = otoliths, FR = fin rays.

^b Sectioned subopercles of least cisco were omitted from the study.

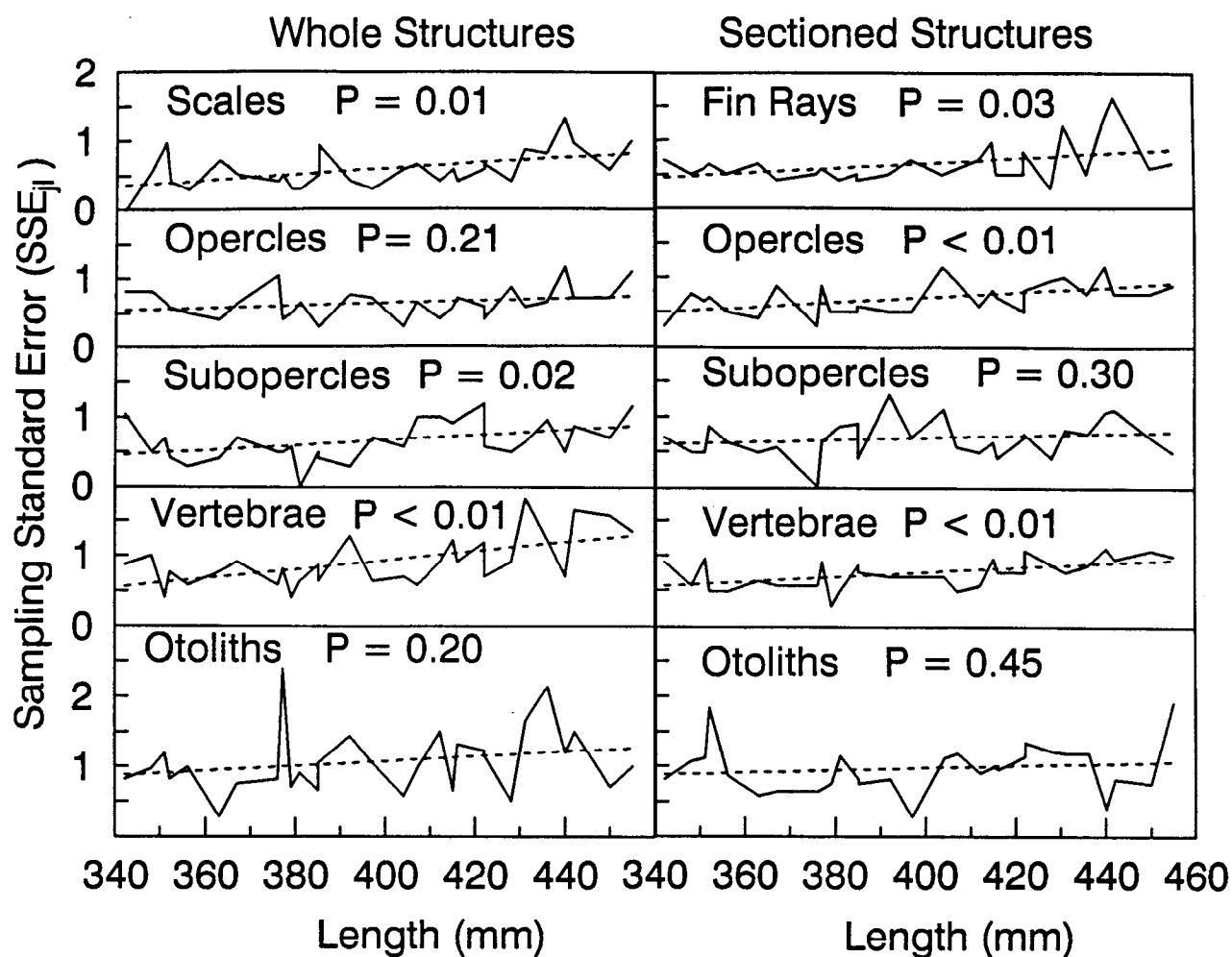


Figure 2. Plots of sampling standard error of structure-fish combinations (SSE_{j1}) against length of humpback whitefish. Dotted lines are the resulting regression lines. Solid lines connect precision values (SSE_{j1}) of individual fish. P -values represent the probability of a linear trend significantly different from zero.

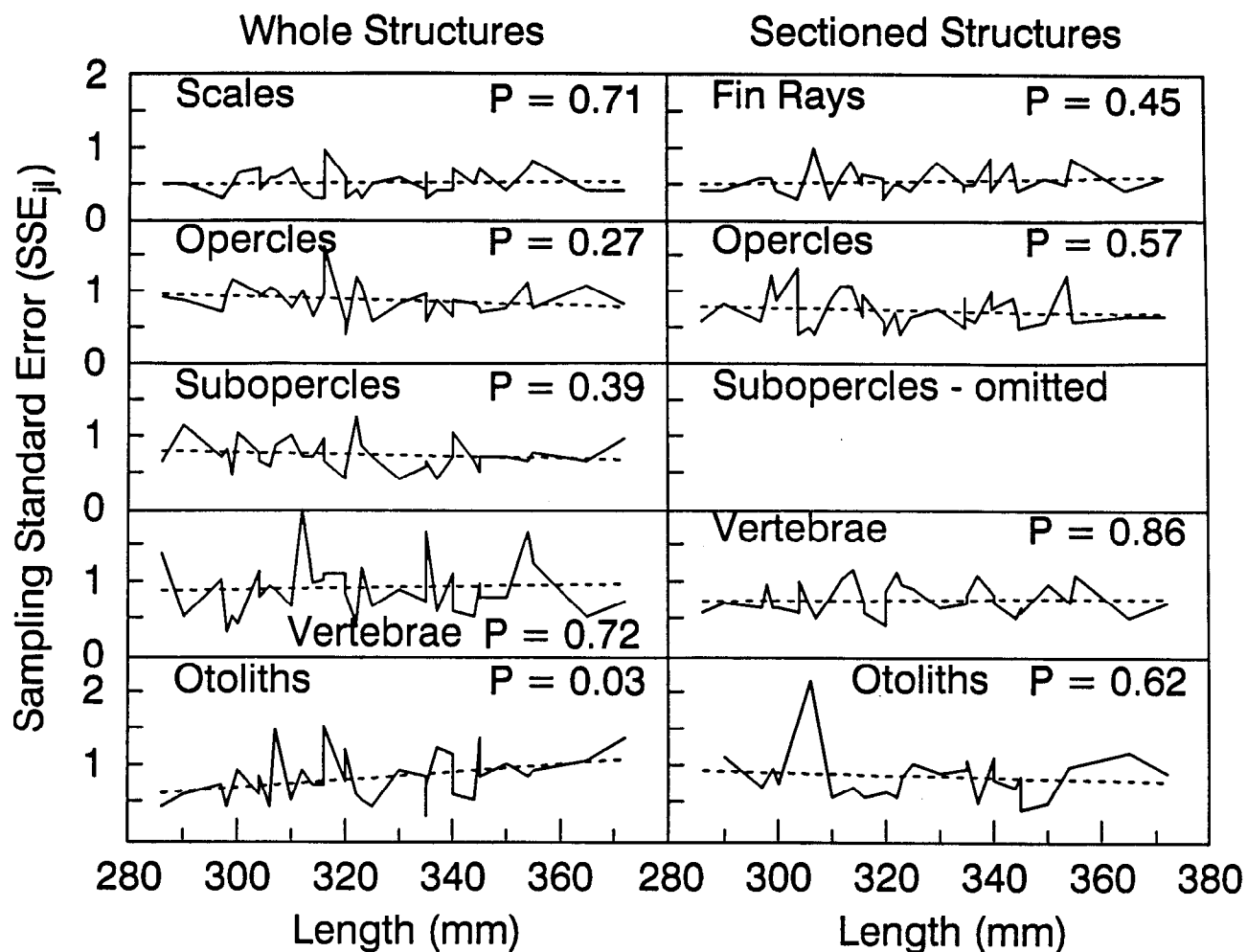


Figure 3. Plots of sampling standard error of structure-fish combinations (SSE_{ij}) against length of least cisco. Dotted lines are the resulting regression lines. Solid lines connect precision values (SSE_{ij}) of individual fish. P-values represent the probability of a linear trend significantly different from zero.

Problems of age determination are often associated with whole structures, scales, and older fish. Some studies have suggested that ages determined from whole rather than sectioned bone structures are underestimates (Beamish 1979; Barber and McFarlane 1987). But ages determined from sections of four types of whitefish bones in this study were not significantly greater than those from corresponding whole structures, sectioned otoliths of least cisco excepted. Sectioning structures resulted in little or no improvement in precision over corresponding whole structures. Other studies have shown bias, particularly underestimation of the age of older fish when scales were used to determine age (Aass 1972; Power 1978; Mills and Beamish 1980). In this study, ages determined from scales were not significantly lower than ages determined from the other structures, and gave the best precision for both species. However, precision decreased with length for several structures, supporting the argument that determining age is increasingly difficult as fish grow older (Aass 1972; Power 1978; Mills and Beamish 1980; Barber and McFarlane 1987).

Ageing fish from their bony structures requires interpretation and subjectivity. Factors such as water conditions, availability of food, and age itself can create ambiguous markings on these structures. Zolotavina and Mukhachev (1976) described false checks on the scales of the pelyad *Coregonus peled* and the broad whitefish *Coregonus nasus*. Related to cessation of feeding when water temperature reached a high in mid-summer, these false markings did not occur annually but occasionally. Accessory checks were also reported by Hogman (1968) on scales of coregonids reared in a laboratory. The accessory checks were formed annually in the fall, and seemed related to an increased protein demand, such as spawning fish might experience. Aass (1972), Powers (1978), and Mills and Beamish (1980) found that problems of age determination were exacerbated in older fish, because as growth slows, annuli either fail to form or become increasingly difficult to distinguish on the outer edges of the structures. The specimens in this study were believed to travel between overwintering grounds in a large wetland (Minto Flats) and a feeding and spawning area in the Chatanika River. Accessory or false checks related to changes in water conditions or spawning could explain the great variation in precision between fish of similar sizes, as well as some of the overall imprecision associated with ages estimated in this study. Crowded markings on the outer edges of bony structures from many of the larger fish, probably caused by slow growth, made annuli difficult to distinguish; precision of age estimates decreased for those fish.

Ignoring precision of age estimates could lead to serious management errors (Beamish and McFarlane 1987). Using data from this study, assume a fishery management agency collects whole otoliths from a sample of humpback whitefish to estimate age composition in year i and again in year $i + 1$. Further, assume the agency uses two staff members to determine ages, one (reader 3 of this study) for fish collected in year i , and the other (reader 4) for those collected in year $i + 1$. Lastly, assume the age composition is identical in year i and year $i + 1$. Many fishery managers, after evaluation of the resulting data (Figure 4), would erroneously conclude that substantial changes in the fish population had occurred, when in fact, such "changes" were entirely due to the effect of precision on the estimation of age composition. If overfishing was considered the cause of these "changes", stricter or more costly regulations could result. Such a scenario could easily occur,

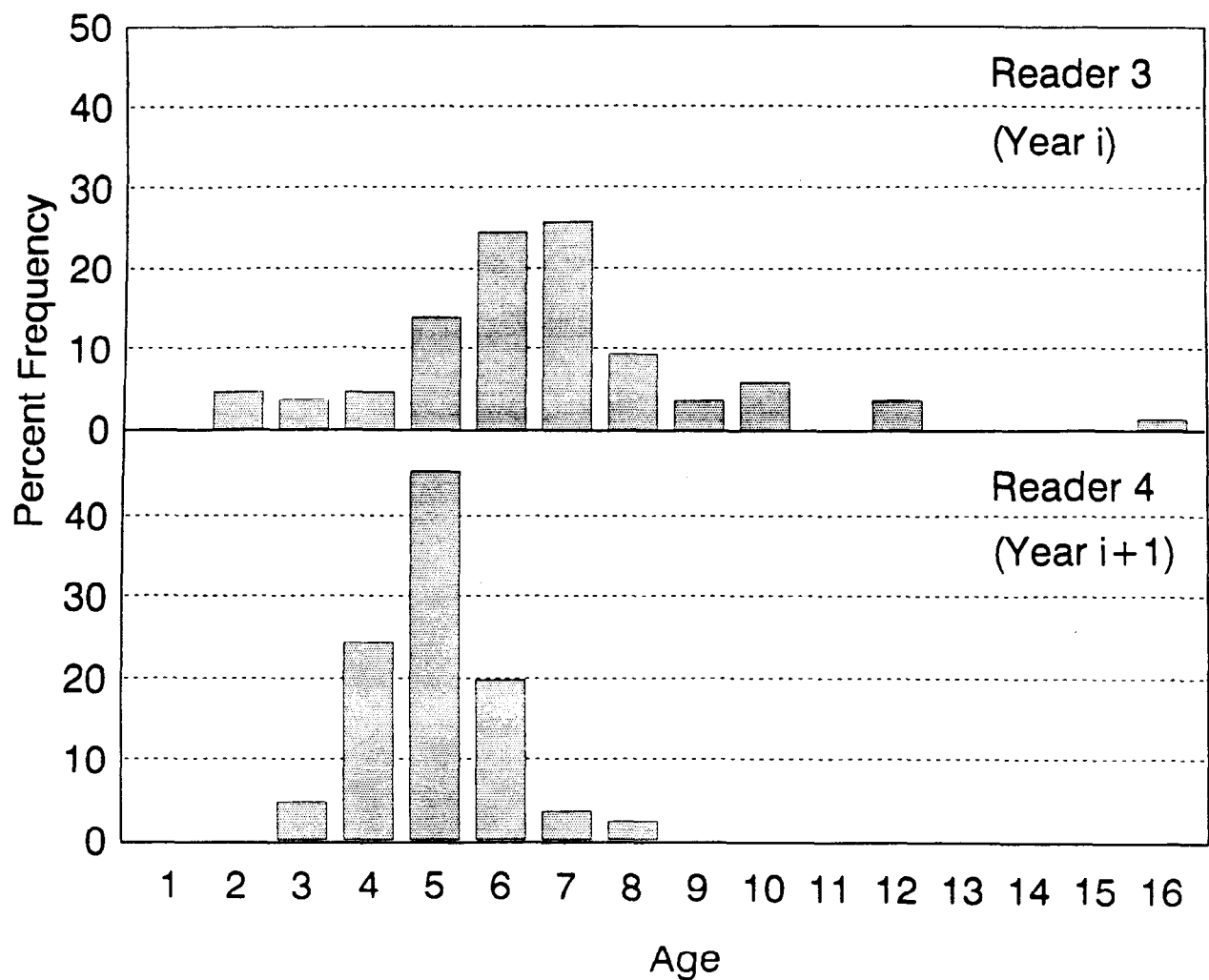


Figure 4. Ages of 29 humpback whitefish determined from whole otoliths three times by reader 3 and reader 4 (year i and $i + 1$ in the hypothetical scenario).

especially when responsibilities for age determination projects are rotated among agency staff. Fishery scientists and managers must consider not only the accuracy, but also the precision, of age determinations of fish.

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